**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| In re Application of | : | Murphy et al. | |
| Serial No. | : | 09/523,809 | Confirmation No. 6553 |
| Art Unit | : | 1633 | |
| Filed | : | March 13, 2000 | |
| Examiner | : | S. Kaushal | |
| For: | | **BIOENGINEERED TISSUE CONSTRUCTS AND METHODS FOR PRODUCING AND USING THEREOF** | |

Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

**SECOND DECLARATION OF KATHERINE C. FARIA**

I, Katherine C. Faria, Director of Process Development at Organogenesis Inc. declare as follows:

1. I am a citizen of the United States of America and currently reside in Middleboro, Massachusetts, U.S.A.

2. I received a Bachelor of Science degree in Biology with a minor in Chemistry in 1993 from University of Massachusetts Dartmouth in Dartmouth, Massachusetts.

3. For over 12 years, I have been engaged in the practice of cell culture. For 8 years, since 1998, with the exception of a 9-month period in 2002, I have been, and currently am, employed at Organogenesis Inc., 150 Dan Road, Canton, MA 02021-2820, in the Process Development department with duties related to culture of human dermal fibroblasts, human epidermal cells; organotypic culture of both single and bi-layer tissue constructs of skin; and media development for these cell cultures and organotypic cell cultures.

4. I have reviewed the above-referenced U.S.S.N. 09/523,809 (the '809 application), including the pending claims, and I am familiar with the subject matter disclosed and claimed therein.

5. I have previously submitted a Declaration of Katherine C. Faria on December 16, 2005, and hereby incorporate my testimony therein.

6. I have reviewed the Final Office Action mailed March 28, 2006 ("*Final Office Action*"), and make this declaration in support of the concurrently filed *37 C.F.R. § 1.114 Response To Office Action.*

7. I understand from reviewing the '809 application that the presently claimed invention is directed to a multilayered cultured skin construct comprising cultured dermal fibroblast cells that synthesize, assemble and produce a layer of extracellular matrix in the absence of any exogenous matrix components and/or a mesh member acting as a support during the culturing conditions. The multilayered skin construct also comprises a second layer of epithelial cells disposed on the first layer to form an epidermal cell layer when the selected epithelial cells are keratinocytes.

8. I understand that in the Office Action, claims 31-71 were finally rejected for failing to comply with the "enablement" requirement of 35 U.S.C. § 112, ¶ 1.

9. I have been informed by the attorneys for the '809 application ("Applicants' attorneys") that the standard for determining whether the specification meets the enablement requirement of 35 U.S.C. § 112, ¶ 1 is whether the experimentation needed to practice the claimed invention is undue.

10. I have been informed by Applicants' attorneys that one of ordinary skill in the art would be an individual with an undergraduate degree in cell biology and at least two years of postgraduate research or work experience in the field of tissue constructs. Thus, in my

opinion, one of ordinary skill in the art, when advised of the teachings of the '809 application, would know the culturing conditions to prepare a layer of dermal fibroblast cells that produce an extracellular matrix having such natural byproducts such as type I and type III collagen, decorin, fibronectin, tenascin, glycosaminoglycans, etc. One of ordinary skill in the art would further know which culturing conditions lead to the formation of epidermis.

11. As such, it is my opinion that one of ordinary skill in the art would understand based on '809 application how to prepare without undue experimentation a multilayered cultured skin construct comprising cultured dermal fibroblast cells that synthesize, assemble and produce a layer of extracellular matrix in the absence of any exogenous matrix components and/or a mesh member acting as a support during the culturing conditions. The multilayered skin construct also comprises a second layer of epithelial cells disposed on the first layer to form an epidermal cell layer when the selected epithelial cells are keratinocytes.

12. The reasons for my conclusion in the preceding paragraph are simple. It is well known that "culturing conditions" is a term of art which include various conditions associated with culturing cells.

13. It is also well known in the art that culturing conditions can be intangible/environmental in nature (*e.g.*, temperature, humidity, etc.) or can be more tangible in nature (*e.g.*, specific additives, growth factors, culture media, supports, etc.).

14. It is further known that the intangible/environmental and the tangible culturing conditions used to culture cells are typically taught or disclosed together in the art. Thus, if the culture media for culturing a specific type of cell is known then all of the other conditions (*i.e.*, support structure, environmental conditions, etc.) would also be known and disclosed in the same art. This is because it is within a scientist's routine practice to disclose what culture media may be used to obtain a layer of dermal fibroblasts or epidermal cell layers

producing their natural byproducts as well as all culturing conditions used to culture the specific cells being researched or experimented on.

15. As explained in the Declaration of Katherine C. Faria filed on December 16, 2005 ("*Faria Declaration*), it is well known what culture media, including defined culture media, could be used to culture dermal fibroblast cells to produce their natural byproducts (*e.g.*, ¶ ¶ 9, 11 and 13 of *Faria Declaration.* Also Specification page 11 line 25 to page 13 line 9, citing U.S. Patent No. 5,712,163, International PCT Publication No. WO 95/31473, a non-patent reference Ham and McKeehan, Methods in Enzymology, 58:44-93, 1979, Examples 4 and 15).

16. Consequently, the culturing conditions to induce dermal fibroblast cells to produce their natural byproducts are all well known and are also disclosed in the '809 application as filed (*e.g.*, Specification, page 12, line 10 to page 19 line 6, Examples 1 and 3).

17. As was also explained in the *Faria Declaration*, it is also well known what culture media could be used to form an epidermal cell layer from keratinocyte cells. Therefore, it is also well known in the art what culturing conditions can be used to form an epidermal cell layer from keratinocyte cells, which are naturally found in such epidermal cell layers and they are also disclosed in the '809 application as filed (*e.g.*, ¶ 12 of *Faria Declaration.* Also Specification page 19, line 18 to page 21 line 11, citing U.S. Patent Nos. 5,712,163, 5,536,656 and 5,374,515, Examples 2, 4, 12 and 16).

18. Indeed, the '809 application provides a number of directions on how to prepare the tissue construct of the invention. For example, it discloses the culturing conditions to grow and expand fibroblast cells (*e.g.*, Specification page 11, line 11, to page 14 line 8, page 14, line 16, to page 18 line 19 and Examples 1, 3, 5, 6, 9-11, 15, 17).

19. The '809 application also discloses the culturing conditions to prepare a layer of extracellular matrix from dermal fibroblast cells in the absence of exogenous matrix

components. (*e.g.*, Specification, page 17, lines 7-28; page 18, line 7 to page 19 line 6; page 23, line 26 to page 24, line 6, Example 1, 3, 5, 6, 9-11, 15, 17 and figure 1).

20. For example, the '809 application teaches that the dermal fibroblast cells are suspended in either base or growth media and are seeded on the cell culture surface at a density between about $1x10^5$ cells/cm$^2$ to about $6.6x10^5$ cells/cm$^2$, more preferably between about $3x10^5$ cells/cm$^2$ to about $6.6x10^5$ cells/cm$^2$ and most preferably at about $6.6x10^5$ cells/cm$^2$ (cells per square centimeter area of the surface). The cells are cultured in growth medium to establish the culture to between about 80% to 100% confluence at which time they are chemically induced to upregulate the synthesis and secretion of extracellular matrix without the use of a synthetic mesh member. (*e.g.*, Specification, page 18, lines 11-17).

21. The '809 application also teaches that the cultures are maintained in an incubator to ensure sufficient environmental conditions of controlled temperature, humidity and gas mixture. Preferred conditions are between about 34 $^0$C to about 38 $^0$C, more preferably 37± 1 $^0$C with an atmosphere between about 5-10 ± 1% $CO_2$ and a relative humidity (Rh) between 80-90%. (*e.g.*, Specification page 17, lines 25-28, Examples 1, 3, 4, 5, 6, 7, 10 etc.).

22. The '809 application further teaches the culturing conditions to prepare a layer of epidermal cells applied on the cell-matrix construct. (*e.g.*, Specification, page 19, line 18 to page 21, line 11, Examples 2, 8, 12 and 16.)

23. For example, the '809 application teaches that keratinocyte cells are seeded onto the cells matrix construct and are then induced to differentiate to form a multilayer epidermis. In other words, the keratinocyte cells are grown by seeding and culturing between about $4.5x10^3$ cells/cm$^2$ to about $5x10^5$ cells/cm$^2$ epithelial cells to the upwardly facing surface of the cell-matrix construct to form a multilayer cell construct. The constructs are then incubated for between about 60 to about 90 minutes at 37 ± 1 $^0$C, 10% $CO_2$ to allow the cells to attach.

(*e.g.*, Specification, page 19 line 18 to page 20, citing U.S. Patent Nos. 5,712,163 and 5,536,656, Examples 2, 4, 8, 12, and 16).

24. The '809 application also provides a number of working examples to practice the claimed invention. The working examples include a variety of different culturing conditions which can be used (*e.g.*, Examples 1, 2, 3, 5, 6, 8, 9, 10, 12, 15, 16, and 17).

25. Thus, as set forth above, the Specification is enabling for the optimization of culturing conditions for inducing human fibroblasts to produce a layer of extracellular matrix in the absence of exogenous matrix components (*e.g.*, page 17, lines 7-24, page 18, 7-19, Examples 1, 3, 5, 6, 9, 10, 15, 17 and figure 1), for inducing the formation of epidermis (*e.g.*, page 19, line 18 to page 21, line 11, citing U.S. Patent Nos. 5,712,163 and 5,536,656, Examples 2, 8, 12 and 16), and for the type of media which can be used at each step during the development of the cultured skin construct as claimed. That is, the Specification teaches to one of ordinary skill in the art each and all the events necessary to assemble the cells into the tissue and how to practice each "event" without undue experimentation. Accordingly, the Specification provides ample guidance for practicing the claimed invention.

26. Armed with this knowledge of the cell culture conditions for which the state of the technology is well known in the art, to prepare a layer of extracellular matrix from dermal fibroblast cells in the absence of exogenous matrix components and to grow human fibroblasts and epidermal cells to form an epidermal layer, one of ordinary skill in the art would understand how to practice the claimed invention. Experimentation, if any is needed, would be routine at most, and certainly would not be undue.

27. Therefore, it is my opinion that one skilled in the art would be able to practice the invention of claims 31-71 without undue experimentation since the '809 application

provides ample teachings of culturing conditions and of type of media which can be used at each step to prepare a cultured skin construct as claimed.

I HEREBY DECLARE that all statements made of my own knowledge are true, and all statements made on information and belief are believed to be true. I make this declaration understanding that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. § 1001) and may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

Date: December 6, 2006 By: _____

Katherine C. Faria